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COMMENTARY

Tailoring Antimicrobial Susceptibility Testing to Individual Species of Coagulase-Negative Staphylococci: Next Up, *Staphylococcus epidermidis*

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ABSTRACT Accurate detection of methicillin resistance among staphylococci is vital for patient care. Methicillin resistance is most commonly mediated by acquisition of the *mecA* gene, which encodes an altered penicillin binding protein, PBP2a. Application of phenotypic methods to detect *mecA*-mediated beta-lactam resistance in staphylococci is becoming more complex as species-specific differences are identified among coagulase-negative staphylococci (CoNS). Previously, interpretative criteria and antimicrobial susceptibility testing (AST) methods specific to the CoNS group were used to evaluate *Staphylococcus epidermidis*. A manuscript by S. N. Naccache, K. Callan, C.-A. D. Burnham, M. A. Wallace, et al. (J Clin Microbiol 57:e00961-19, 2019, https://doi.org/10.1128/JCM.00961-19) details experiments revealing that *S. epidermidis*, the most common clinically isolated CoNS, requires tailored use of previously described methods and interpretive criteria to reliably identify the presence of *mecA*-mediated methicillin resistance.

Staphylococci have historically been divided into two broad groups based on the potential to cause clinical infections in humans and the detection of coagulase production from cultured isolates. These two groups consist of (i) the more pathogenic, coagulase-positive *Staphylococcus aureus* group and (ii) the less virulent, diverse coagulase-negative staphylococci (CoNS). The lines between these two groups continue to be blurred with time as we increasingly identify coagulase-positive staphylococci seen in veterinary practice causing human infections or further understand the differences in pathogenesis between individual species encompassing the CoNS (1).

Overall, the CoNS are a heterogeneous group of species that share similarities in that they are part of the regular skin and mucous membrane microbiota of humans and animals and are opportunistic pathogens (2). For this reason, they have classically been treated as a single entity clinically and within clinical microbiology laboratories. This was a logical method of approaching these organisms when the clinical microbiology laboratory had to rely on biochemical tests for differentiating the individual species. However, with the introduction of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and the near-replacement of automated biochemical systems for identification of most bacteria over the past decade, we have the capability of more simply and rapidly differentiating species within this genus. As the ability to differentiate these organisms has improved, differences within the species have become more apparent. There are differences in the rates of antimicrobial resistance and pathogenicity and in the ability to form biofilms (2, 3). These differences have raised the issue of whether these species require individualized approaches for antimicrobial susceptibility testing (AST).

S. epidermidis is a prototypic CoNS and is arguably the most significant of the CoNS (2). *S. epidermidis* is part of the skin microbiota of healthy individuals and typically lives in the moist skin areas. It is the CoNS species most commonly recovered from clinical

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specimens (2) and is an important cause of nosocomial and foreign-body-related infections, especially among immunocompromised individuals (4). Because of the ability of this species to form biofilms, treatment can be complicated (5) and may require protracted courses of antimicrobials. Furthermore, *S. epidermidis* isolates often display resistance to many classes of antimicrobials, including the beta-lactams (6).

In performing AST on species of the genus *Staphylococcus*, it is vital to determine whether the organism harbors methicillin resistance. Methicillin resistance is most commonly mediated by acquisition of the *mecA* gene, which encodes an altered penicillin binding protein (PBP2a or PBP2′). Since PBP2a provides resistance to almost all beta-lactam antimicrobials (with the exception of ceftaroline), it is of utmost importance to accurately determine methicillin resistance status. In most species, cefoxitin resistance has been found to be a better marker for *mecA*-mediated resistance than oxacillin itself, so cefoxitin is often used as a surrogate agent for oxacillin (7).

The need for individualized AST methods and breakpoints for CoNS species was first shown with *S. lugdunensis*. *S. lugdunensis* isolates positive for *mecA* were found to have oxacillin MICs of $\geq$4 μg/ml consistent with the higher breakpoint set for *S. aureus* (8–10). This is not surprising, given that *S. lugdunensis*, while classified as CoNS, behaves clinically in a manner similar to that seen with *S. aureus* (2). After this, other *Staphylococcus* species were evaluated. In 2016, it was discovered that *S. pseudintermedius*, a coagulase-positive veterinary species which had a high potential for misidentification as *S. aureus* prior to the introduction of MALDI-TOF MS, required the use of the veterinary oxacillin MIC and disk diffusion breakpoints (previously described in the CLSI VET01-S2 supplement) to more accurately predict the presence of methicillin resistance than those set for human clinical CoNS and *S. aureus* isolates (10–12). They also determined that cefoxitin disk diffusion or MIC methods should not be used as a surrogate for oxacillin methods, as they could not reliably detect *mecA*-mediated resistance in this species. In 2018, further studies suggested that the recommendations and breakpoints set for *S. pseudintermedius* were also ideal for the veterinary coagulase-variable species *S. schleiferi* (1). Furthermore, these studies found that cefoxitin disk diffusion should not be used as a method for confirming that non-*S. epidermidis* CoNS isolates from serious infections with oxacillin MICs in the 0.5-to-2.0-μg/ml range are truly oxacillin resistant. As such, laboratories should confirm the susceptibility of such isolates by *mecA* nucleic acid amplification test or PBP2a test (1, 12).

In this issue, Naccache et al. detail experiments that they used to evaluate phenotypic methods and breakpoints to determine *mecA*-mediated methicillin resistance in contemporary clinical *S. epidermidis* isolates (6). Methicillin resistance has been observed in up to 90% of *S. epidermidis* isolates which may demonstrate heteroresistance (6, 13). Therefore, accurate knowledge of the AST profile, including the presence or absence of *mecA*, is clinically important. The authors showed that the best methods to determine the presence of *mecA*-mediated resistance in *S. epidermidis* were oxacillin disk diffusion testing with the *S. pseudintermedius*/*S. schleiferi* M100-S28 interpretations and oxacillin MIC or cefoxitin disk diffusion with the M100-S28 CoNS interpretations (14). Furthermore, they found the performance of PBP2a assays following the manufacturer's instructions (with or without oxacillin induction based on the specific test) was reliable compared to *mecA* PCR. The results from these studies were used to update the approved methods and interpretive criteria to determine cefoxitin and oxacillin results among *S. epidermidis* isolates in the newest edition of the CLSI M100-S29 document (15). Due to the increasing complexities of testing CoNS for *mecA*-mediated resistance, the CoNS designation has been removed from most of the M100-S29 document and replaced with language to reflect species-dependent testing for oxacillin and cefoxitin (15).

In conclusion, it is becoming more important that clinical microbiology laboratories have the capability to further distinguish the CoNS to the species level due to the increasing complexity of AST methods and associated interpretations that are species dependent. Since *S. epidermidis*, which makes up the largest proportion of clinically relevant CoNS, requires tailored methodologies for AST, there is a strong suggestion

that the other non-*epidermidis* CoNS cannot be evaluated as a single group. Instead, there needs to be evaluation of the best methods of AST for each of the CoNS, and each of these species may need to be handled differently clinically. We look forward to future work on the optimization of AST for other CoNS. Which species is up next?

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